

VALIDATION RESULTS FOR METHOD NO. Covance Harrogate 2001-043-D1					
		MEAN	RELATIVE STD. DEV. (%)	RANGE	SEE (conc)
ANALYTE	Nicotine-N- β -glucuronide				
MATRIX	Human urine				
DATE	19-Oct-01				
LLOQ	1 ng/mL. Accuracy 95.0%. RSD (Pre- or Post- Calibration): 15.4%				
STANDARD ERROR OF ESTIMATE OF QC SAMPLES (concentration units) (SEE = S.D./SQRT (# of determinations))					
	2 ng/mL	Low QC			0.059
	400 ng/mL	Med QC			4.158
	750 ng/mL	High QC			6.886
DESCRIPTIVE INFORMATION ON INSTRUMENT RESPONSE					
Function (Linear, Quadratic, etc.)	Linear				
Slope (mean)	0.0515				
Intercept (mean)	0.03890				
Other (mean regression value)	0.9984 (1/x ² weighted)				
RECOVERY (as a %)					
High QC in (matrix)=	not assessed				
Med QC in (matrix)=	not assessed				
Low QC in (matrix)=	not assessed				
Results with different sample amounts (results of QC evaluations) intra-assay					
LLOQ QC=	1 ng/mL	0.85	15.4%	0.64-1.00	0.053
Low QC=	2 ng/mL	1.84	19.0%	1.40-2.37	0.142
Med QC=	400 ng/mL	339.93	2.3%	331.03-351.68	3.187
High QC=	750 ng/mL	658.14	2.4%	628.95-671.74	6.419
Dilution QC1=	2000 ng/mL	1738.96	4.5%	1621.3-1825.1	31.832
Dilution QC2=	20000 ng/mL	19175.1	4.5%	17987-20288	355.878
Results with different sample amounts (results of QC evaluations) inter-assay					
LLOQ QC=	n/a	n/a	n/a	n/a	n/a
Low QC=	2 ng/mL	1.84	12.7%	1.40-2.37	0.059
Med QC=	400 ng/mL	347.66	5.1%	315.69-382.28	4.158
High QC=	750 ng/mL	660.46	4.4%	610.82-738.78	6.886
STABILITY (assuming any degradation is linear):					
a) Room Temperature in Matrix (+/- %/24 hrs)		Stable			
b) Freeze/thaw (+/- % /3 cycles)		Stable			
c) Processed sample (+/- %/24 hrs)	not assessed	% maximum			

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Footnotes from SOP 66 Rev 1: Batch annotation

AS = Above data system
 AR = Anomalous result
 BPS = Below data system
 CO = Confirms original result
 DNU = Data not used
 DNR = Data not required
 HI = High internal standard
 IM = Instrument malfunction (add reason if known)
 IP = Interferent peak
 IS = Insufficient sample
 LI = Low internal standard
 M = This result is a median value
 ME = Mis-extraction
 MI = Mis-injection
 NR = No result
 NS = No sample taken/received
 OR = Over range
 PC = Poor chromatography
 PF = Poor fit with rest of regression (excluding standard improves the correlation coefficient / curve fit)
 P = Poor fit, calibration standard outside acceptance criteria
 PP = Positive predose
 PS = Poor sensitivity
 SA = Sample dropped/spilt/spoilt during preparation (Lab accident)
 = Spiking error
 LR = Out of range (when LI OR raised)

Values in parentheses are not used in calculations

TABLE 1

Study no. 0044-020

Validation for the determination of nicotine-N- β -glucuronide in human urine:
Intra-assay precision and accuracy data for nicotine-N- β -glucuronide quality control samples

Replicate	QC 1 ng/mL (LLOQ QC)	QC 2 ng/mL (LoQC)	QC 400 ng/mL (MeQC)	QC 750 ng/mL (HiQC)	QC 2000 ng/mL (Di QC 1)*	QC 20000 ng/mL (DiQC 2)**
	Observed concentration (ng/mL)	Observed concentration (ng/mL)	Observed concentration (ng/mL)	Observed concentration (ng/mL)	Observed concentration (ng/mL)	Observed concentration (ng/mL)
1	0.78	1.80	343.72	666.48	1800.76	19924.57
2	0.81	1.63	351.68	668.44	1621.31	18425.94
3	0.64	1.40	343.14	653.53	1825.08	20288.14
4	1.00	1.71	337.69	659.68	1690.50	17986.72
5	0.94	2.11	331.03	628.95	1710.58	19126.18
6	0.92	2.37	332.33	671.74	1785.52	19327.31
Mean (ng/mL)	0.85	1.84	339.93	658.14	1738.96	19179.81
Standard deviation (n-1)	0.131	0.349	7.807	15.724	77.973	871.720
Precision (%)	15.4	19.0	2.3	2.4	4.5	4.5
Accuracy (%)	85.0	92.0	85.0	87.8	86.9	95.9

* analysed after 5-fold dilution with blank human urine

** analysed after 50-fold dilution with blank human urine

TABLE 2

Study no. 0044-020

Validation for the determination of nicotine-N- β -glucuronide in human urine:
Inter-assay precision and accuracy data for nicotine-N- β -glucuronide quality control samples

Batch	Replicate	QC 2 ng/mL (LoQC)	QC 400 ng/mL (MeQC)	QC 750 ng/mL (HiQC)
		Observed concentration (ng/mL)	Observed concentration (ng/mL)	Observed concentration (ng/mL)
HUVAl001RI	1	1.80	343.72	666.48
	2	1.63	351.68	668.44
	3	1.40	343.14	653.53
	4	1.71	337.69	659.68
	5	2.11	331.03	628.95
	6	2.37	332.33	671.74
HUVAl002RI	1	3.10	371.88	653.53
	2	1.61	364.39	688.75
	3	1.61	382.28	678.54
	4	1.76	337.89	738.78
	5	1.81	351.54	674.66
	6	1.83	375.68	683.14
HUVAl003	1	3.46	357.11	610.82
	2	1.92	324.44	669.75
	3	1.79	346.68	641.97
	4	1.96	315.69	626.22
	5	2.00	346.94	633.68
	6	2.07	343.80	639.62
Mean (ng/mL)		2.00 (1.84)	347.66	660.46
Standard deviation (n-1)		0.520 (0.234)	17.641	29.215
Precision (%)		26.0 (12.7)	5.1	4.4
Accuracy (%)		100.0 (92.0)	86.9	88.1
n		18 (16)	18	18

* Values in parentheses calculated omitting anomalous/outlying result

TABLE 3

Study no. 0044-020

Validation for the determination of nicotine-N- β -glucuronide in human urine:

Inter-assay precision and accuracy data for nicotine quality control samples

Batch	Replicate	QC 2 ng/mL (LoQC)	QC 400 ng/mL (MeQC)	QC 750 ng/mL (HiQC)
		Observed concentration (ng/mL)	Observed concentration (ng/mL)	Observed concentration (ng/mL)
HUVAL001RI	1	1.66	375.42	706.35
	2	1.89	401.33	705.75
HUVAL002RI	1	2.22	367.40	867.78
	2	1.76	422.77	745.00
HUVAL003	1	1.86	339.34	727.43
	2	1.97	353.52	632.53
Mean (ng/mL)		1.89	376.63	730.81
Standard deviation (n-1)		0.193	30.823	77.277
Precision (%)		10.2	8.2	10.6
Accuracy (%)		94.5	94.2	97.4
n		6	6	6

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TABLE 4

Study no. 0044-020

Validation for the determination of nicotine-N- β -glucuronide in human urine:
Precision and accuracy data for nicotine quality control samples taken
through the enzymatic deconjugation procedure

Batch	Replicate	QC 2 ng/mL (LoQC)	QC 400 ng/mL (MeQC)	QC 750 ng/mL (HiQC)
		Observed concentration (ng/mL)	Observed concentration (ng/mL)	Observed concentration (ng/mL)
HUVAL002RI	1	1.74	417.82	700.88
	2	1.91	405.54	718.00
	3	2.43	386.25	783.27
	4	1.90	405.90	752.19
	5	2.07	405.56	792.15
	6	1.89	449.10	766.22
Mean (ng/mL)		1.99	411.70	752.12
Standard deviation (n-1)		0.240	20.944	36.225
Precision (%)		12.1	5.1	4.8
Accuracy (%)		99.5	102.9	100.3
n		6	6	6

TABLE 5

Study no. 0044-020

Validation for the determination of nicotine-N- β -glucuronide in human urine:
Precision and accuracy data for mixed (glucuronide/aglycone; 50:50 w/w) quality
control samples taken through the enzymatic deconjugation procedure

Batch	Replicate	QC 2 ng/mL (LoQC)	QC 400 ng/mL (MeQC)	QC 750 ng/mL (HiQC)
		Observed concentration (ng/mL)	Observed concentration (ng/mL)	Observed concentration (ng/mL)
HUVAL002RI	1	1.77	353.12	691.93
	2	1.83	388.82	704.90
	3	1.78	377.30	662.13
	4	1.52	390.42	730.69
	5	1.75	390.09	669.24
	6	1.76	369.73	692.88
Mean (ng/mL)		1.74	378.25	691.96
Standard deviation (n-1)		0.109	14.866	24.796
Precision (%)		6.3	3.9	3.6
Accuracy (%)		87.0	94.6	92.3
n		6	6	6

TABLE 6

Study no. 0044-020

Validation for the determination of nicotine-N- β -glucuronide in human urine:
Inter-assay precision and accuracy data for calibration standards

Batch	Back-calculated concentrations (ng/mL)										Curve parameters		
	Calibration level (ng/mL)										Gradient (m)	Intercept (c)	Correlation coefficient (r) *
	1	2	5	10	50	100	250	500	900	1000			
IUVAL001RI	1.06	1.80	4.86	9.77	50.95	98.37	254.80	497.66	920.62	1054.44	0.0531	0.0482	0.9986
IUVAL002RI	1.05	1.89	4.81	8.91	51.46	101.03	246.46	536.19	970.24	981.17	0.0476	0.0481	0.9979
IUVAL003	0.99	2.12	4.64	16.78 PF	50.31	95.36	252.00	504.16	964.71	980.50	0.0537	0.0203	0.9987
Mean (ng/mL)	1.03	1.94	4.77	9.34	50.91	98.25	251.09	512.67	951.86	1005.37	-	-	-
Standard deviation (n-1)	0.038	0.165	0.115	-	0.576	2.837	4.244	20.627	27.193	42.497	-	-	-
Precision (%)	3.7	8.5	2.4	-	1.1	2.9	1.7	4.0	2.9	4.2	-	-	-
Accuracy (%)	103.0	97.0	95.4	93.4	101.8	98.3	100.4	102.5	105.8	100.5	-	-	-

* the coefficient of determination (r^2) is the square of the correlation coefficient

PF = Poor fit, calibration standard outside acceptance criteria

TABLE 7

Study no. 0044-020

Validation for the determination of nicotine-N- β -glucuronide in human urine:
Room temperature stability of nicotine-N- β -glucuronide in human urine

Quantity control level (ng/mL)		Replicate Deconjugated QC samples		Baseline QC samples (no deconjugation)		QC samples stored for 24 h prior to extraction (no deconjugation)	
		Observed Peak Area Ratio	Mean	Observed Peak Area Ratio	Mean (Aglycone %)	Observed Peak Area Ratio	Mean (Aglycone %)
2	1	0.2058	0.1384	0.0368	0.0454 (32.8)	0.0366	0.0483 (34.9)
	2	0.1234		0.0859		0.0444	
	3	0.1163		0.0318		0.0497	
	4	0.1257		0.0386		0.0527	
	5	0.1277		0.0435		0.0493	
	6	0.1317		0.0360		0.0568	
750	1	32.8152	34.2216	0.5787	0.5699 (1.7)	0.5372	0.6091 (1.8)
	2	35.9792		0.5922		0.6908	
	3	34.4879		0.5284		0.6206	
	4	33.6425		0.5679		0.6268	
	5	34.0428		0.5960		0.5394	
	6	34.3617		0.5564		0.6398	

TABLE 8

Study no. 0044-020

Validation for the determination of nicotine-N- β -glucuronide in human urine:
Freeze/thaw stability of nicotine-N- β -glucuronide in human urine

Quality control level (ng/mL)	Replicate	Deconjugated QC samples		Baseline QC samples (no deconjugation)		QC samples subjected to 3 additional freeze/thaw cycles prior	
		Observed Peak Area Ratio	Mean	Observed Peak Area Ratio	Mean (Aglycone %)	Observed Peak Area Ratio	Mean (Aglycone %)
2	1	0.2058	0.1384	0.0368	0.0454 (32.8)	0.0180	0.0272 (19.7)
	2	0.1234		0.0859		0.0247	
	3	0.1163		0.0318		0.0240	
	4	0.1257		0.0386		0.0376	
	5	0.1277		0.0435		0.0372	
	6	0.1317		0.0360		0.0218	
750	1	32.8152	34.2216	0.5787	0.5699 (1.7)	0.6141	0.6061 (1.8)
	2	35.9792		0.5922		0.5848	
	3	34.4879		0.5284		0.5355	
	4	33.6425		0.5679		0.7137	
	5	34.0428		0.5960		0.5345	
	6	34.3617		0.5564		0.6538	

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TABLE 9

Study no. 0044-020

Validation for the determination of nicotine-N- β -glucuronide in urine area:
Stability of stock solutions stored at nominal 4°C for X days

Date of stock solution preparation	Replicate	Peak area of stock solution.	Mean peak area value	% Difference of stored solution from new solution
xx/xx/xx (new)	1		#DIV/0!	#DIV/0!
	2			
	3			
	4			
	5			
	6			
xx/xx/xx (stored)	1		#DIV/0!	
	2			
	3			
	4			
	5			
	6			